OIPE IAP61
JUL 20 2006
PATENT & TRADEMARK OFFICE

07-21-06. IFW 1743.

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
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Total Number of Pages in This Submission: 14

Application Number	10/068,663
Filing Date	Feb. 6, 2002
First Named Inventor	Chuan Li
Art Unit	1743
Examiner Name	Maureen M. Wallenhorst
Attorney Docket Number	

ENCLOSURES *(Check all that apply)*

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Remarks

Additional three pages of suppliment documents are included (not numbered).

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Chuan Li		
Signature			
Printed name	Chuan LI		
Date	July 20, 2006	Reg. No.	

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Amendments to the application PROTEIN STANDARD FOR ESTIMATING SIZE AND MASS

Applicant Name: Chuan Li

Date: July 20, 2006

Application/Control Number: 10/068,663

Art Unit: 1743

a.) Introductory Comments

Mizutani (US patent no. 3,880,814) uses a sample solution containing 15 mg of conalbumin having a molecular weight of 87,000, 4 mg of ovalbumin having a molecular weight of 46,000 and 7 mg of lysozyme having a molecular weight of 14,600 on an invented column to demonstrate that the column can separate these polypeptides. Mizutani fails to teach that these polypeptides together can be used as a protein standard to determine the size and quantity of a protein simultaneously. The obvious question is if this sample solution can be used as quantitative protein standard to determine protein size and quantity on a gel. The answer is definitely no. The disclosed quantitative protein standard is not a simple sample solution of three polypeptides of different sizes and amounts. While sizes of polypeptides can be accurately determined by various means, quantities of polypeptides are different. Each polypeptide has a unique amino acid composition and therefore has unique dye binding property. It is well documented that same amount of different polypeptides give different staining intensities by a staining assay. The staining intensities can be 3 to 5 times different. It is also observed that different polypeptides with same staining intensity can contain different amount of protein. The amount of protein can be 5 to 10 times different. In addition, there are many assays to determine protein quantity. Each assay can be a few times different than another because of different mechanisms used in the quantification. Furthermore, each protein preparation may contain different amount of various chemicals. Some of these chemicals may interfere with a given protein quantification assay. Therefore same amount of a polypeptide of different preparations may give different quantities with same protein assay. Because of these problems, it is not possible to use a simple mixture of three polypeptides of different sizes and amounts as a protein standard to determine the size

and quantity of a protein on a gel. Conalbumin, ovalbumin and lysozyme each has different amino acid composition. Their quantity may be determined by different protein assays. Each protein preparation contains different composition and amount of detergents, buffers and salts. For example, when 15, 4, and 7 mg of conalbumin, ovalbumin and lysozyme are mixed together, the ratio of their staining intensities by a given assay cannot be 15:4:7. It may be 3:5:10 when the third protein stains strongest and the first protein stains weakest. It may also be 20:5:1 when the first protein stains strongest and the third protein stains weakest. The obtained staining intensity ratio can be any unpredictable number. It is impossible to obtain a ratio of 15:4:7 which reflect the amounts of these proteins. Therefore the sample solution taught by Mizutani can only be used as a protein size standard but not a quantity standard.

Now the question is if the claimed invention is anticipated by Mizutani's teaching. The applicant believes the claimed invention is not anticipated by Mizutani for those skilled in the art at the time the invention was made. The reasons are following:

1. The facts that the claimed invention is not a simple mixture of polypeptides of different sizes and amounts indicate the application is not anticipated by Mizutani.

2. The patent of Mizutani is granted on April 29, 1975 which is over 30 years ago. Because of obvious advantages of saving labor and cost with the claimed invention, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for over 30 years indicates the claimed invention is not anticipated by Mizutani.

3. The applicant devised new principles of operation to solve the problems in previous paragraphs in the amendments: (1) The absolute amount of each protein is not relevant in the quantitative protein standard. (2) The relative staining intensity of each protein will represent its quantity against a standard protein such as BSA or lysozyme. In other words, the relative amount of protein obtained from the relative staining intensity does not reflect the amount of each protein in the quantitative protein standard but represent the relative amount of protein of the standard protein such as BAS or lysozyme. (3) The staining assay used to prepare the quantitative protein standard should be similar to the assay using the protein standard to make the quantification reliable and consistent. This means that if the quantitative protein standard is made by Coomassie Blue staining,

the standard should only be used by Coomassie Blue staining assay. Using it in silver staining assay will not be accurate in estimating the protein amount. Similarly if UV spectrometer is used to quantify the proteins in the quantitative protein standard, it cannot be used in either Coomssie Blue staining or silver staining to accurately estimate the amount of a sample protein. (4) Same experimental procedures should be used for estimating the amount of proteins in the quantitative protein standard and using the standard. The amount of the proteins in the quantitative standard is estimated by a polyacrylamide gel electrophoresis followed by a detection assay such as Coomassie Blue staining since the standard is to be used in the same procedures. Electrophoresis on a polyacrylamide gel before a detection assay to estimate the amount of protein in the protein standard is a critical step. Substances such as detergents, buffers, salts and reducing agents that may affect detection are separated during electrophoresis and washed out during staining and de-staining procedures. The quantitative protein standard can be made accurate only with all these procedures. Estimating protein amount of quantitative protein standard without electrophoresis step will not be accurate even same assay is used in using the standard. Each protein contains different detergents, buffers, salts and reducing reagents. These substances may affect quantity estimation with a given detection assay.

The quantitative protein standard was made possible only after all of these new principles of operation were used. Involvement of new principles of operation indicates the disclosed invention is not obvious. These new principles of operation are inventive steps of the disclosed invention. It is not possible to perform the disclosed invention without any of these inventive steps.

4. The invention solves a long-felt, long-existing, but unsolved need. The applicant has been working on protein sizing and quantification for many years. It was always painful for the applicant to use the laborious method taught by Fishbein similar as that taught by Houghton et al (cited in the previous Office Actions). This laborious and costly method is used today in many academic and industrial labs as revealed recently by Houghton et al. Therefore solution to the long-felt and unsolved need further indicates the application is not anticipated by Mizutani.

5. The protein standard from the disclosed invention has attained commercial acquiescence. Recently, the patent assignee Expression Technologies Inc. has put the protein standard information on its web site for testing sales. Scientists from both academic labs and biotech companies are purchasing the protein standard with prices two to five times of regular protein size standards. A copy of recently paid invoice and regular protein size standards from other two established reagent companies (Invitrogen and BioRad) are enclosed with the amendments. Comparable unstained size standards from these companies are highlighted. Our quantitative protein standard sold at two to five times the price of the size standards from the well established biotech reagent companies and that no quantitative protein standard available for over 30 years indicate the application is not anticipated by Mizutani.

Claims 26-28 are cancelled. Rewritten claims are in independent form with all the limitations of the base claim and any intervening claims.

The amendments to the specification are presented in the correct format. Entire paragraphs in Examples 1, 2 and 3 which the insertions are made are repeated in the amendments

In conclusion, the disclosed invention involves new principles of operation and it is not anticipated by Mizutani. Claims 21-38 are cancelled. New Claims 39-55 are rewritten to reflect the new principles of operation of the disclosed invention. Therefore the re-written claims are submitted that patentable subject matter is clearly presented. If the examiner agrees but does not feel that the present claims are technically adequate, applicant respectfully requests that the examiner write acceptable claims pursuant to MPEP 707.07(j).